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# Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen

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**Abstract**

This work demonstrates that spermatozoa from five avian species (chicken, turkey, guinea fowl, duck and goose) are all characterised by high proportions of polyunsaturated fatty acids, from 46 (turkey) to 55% (duck) of total. For each of the species, the most abundant fatty acids were arachidonic (20:4n–6) and docosatetraenoic (22:4n–6) acids, representing between 22 (turkey) and 40% (chicken) of total. Significant activities of the major isozymes of superoxide dismutase and glutathione peroxidase, which protect against the peroxidation associated with high degree of fatty acid unsaturation, were found in spermatozoa from all species. The seminal plasma also had these activities and showed additional mechanisms for protecting spermatozoa from peroxidation. In general terms, these lipid and enzyme proteins were similar between the five avian species and different from those reported for mammalian sperm. 

*Keywords:* Chicken; Turkey; Drake; Gander; Guinea fowl; Spermatozoa; Phospholipids; Antioxidants

## 1. Introduction

Chicken and turkey spermatozoa are characterised by very high proportions of long chain polyunsaturated fatty acids (PUFAs), mainly 20:4n–6 and 22:4n–6 [22,36], the ratio of polyunsaturated to saturated fatty acids in chicken and turkey spermatozoa being 0.9 and 1.1, respectively [36]. In comparison, mammalian spermatozoa contain much higher levels of n–3 polyunsaturates, particularly docosahexaenoic acid (22:6n–3), and in general terms are more unsaturated compared to avian species [24,29,33,35].

Despite the lower amounts of PUFAs in avian sperm compared to mammalian sperm, they have higher amounts of PUFAs than other avian tissues and are nonetheless susceptible to peroxidation [15,16,42]. In fact, lipid peroxidation is considered to be a major cause of fertility loss during avian sperm storage and other in vitro manipulations [11], as has been shown for mammalian spermatozoa [1–4,6,19–21].

Because of the seriously damaging potential of reactive oxygen species, cells depend on elaborate defence mechanisms to effectively neutralise or metabolise these toxic intermediates and to prevent significant free radical injury. Antioxidant systems of cells include fat-soluble (vitamin E, carotenoids and ubiquinones) and water soluble (ascorbic acid, glutathione and uric acid) natural antioxidants, and antioxidant enzymes: glutathione peroxidase (GSH-Px; EC 1.11.1.9); catalase (EC 1.11.1.6); and superoxide dismutase (SOD; EC 1.15.1.1) [43], including both the mitochondrial, manganese-de-

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pendent form (Mn-SOD) and the cytoplasmic copper/zinc (Cu,Zn-SOD) forms [31]. The first line of defence against the superoxide radicals are the SODs. Hydrogen peroxide formed during superoxide dismutation is highly toxic to the spermatozoa [5,13] and is removed by GSH-Px and catalase [18]. GSH-Px is more comprehensively active compared to catalase in that its selenium-dependent form is able to destroy both lipid hydroperoxides and hydrogen peroxide [28].

Despite the importance of SOD and GSH-Px in the protection of cells against lipid peroxidation, their activity in avian sperm has received little attention. When SOD activity in spermatozoa from boar, rabbit, stallion, donkey, ram, bull, man and chicken was compared, donkey sperm had the highest and chicken spermatozoa the lowest activity [30]. In a comparison of chicken and turkey spermatozoa, chicken spermatozoa contained 1.48, while turkey spermatozoa contained 0.32 activity units per $10^9$ cells, and turkey sperm SOD activity was reduced to 28% by 1 mM KCN, while chicken sperm SOD activity was reduced to 66% [14]. The presence of GSH-Px in chicken sperm has also been confirmed [22,40].

High levels of PUFAs may promote lipid peroxidation and limit the viability of chicken and turkey spermatozoa. Little is known about the fatty acid composition of other avian spermatozoa and even less detail is known about antioxidant processes in avian spermatozoa. This report, therefore presents results of a comparative study of the fatty acid composition of the spermatozoa, SOD and GSH-Px activities in the spermatozoa and seminal plasma, and total antioxidant activity in the seminal plasma of the chicken (*Gallus gallus domesticus*), turkey (*Meleagris gallapavo*), guinea fowl (*Numida melagris)*, duck (*Anas platyrhynchos*) and goose (*Anser anser*).

## 2. Materials and methods

Male chickens, turkeys, guinea fowls, geese, ducks were from commercial lines. All birds were housed in individual battery cages and fed standard commercial diets formulated for each species before and during the experimental period. Upon sexual maturity (age: 25 weeks in chickens, 26–29 weeks in guinea fowl and ducks, 35 weeks in geese and 45 weeks in turkeys), semen was routinely collected two or three times a week, depending on species, by massage [8], except for ducks for which female teasers were used. Within each species, ten individual ejaculates, collected on the same day, were prepared and analysed. The concentrations of spermatozoa in each ejaculate were measured using a calibrated photometer (wavelength 535 nm). Spermatozoa were separated from seminal plasma by centrifugation ($500 \times g$, 10 min) and the resultant sperm pellets were frozen for further analyses. Seminal plasma was prepared by successive centrifugations as previously described [9] and then frozen for further analyses.

Spermatozoa were extracted for total lipid by standard procedures following homogenisation in chloroform/methanol (2:1, v/v) [12]. Portions of the total lipids were subjected to transmethylation by refluxing with methanol:toluene:sulphuric acid (20:10:1, v:v:v) in the presence of a pentadecanoic acid standard [12]. The composition of the resultant fatty acid methyl esters was determined by gas-liquid chromatography using a capillary column system (Carbowax, 30 m $\times$ 0.25 mm, film thickness 0.25 $\mu$m; Alltech, Carnforth, UK) in a CP9001 instrument (Chrompack, Middleburg, Netherlands). Integration of the peaks and subsequent data handling were performed using an EZ Chrom Data System (Scientific Software, San Jose, CA) enabling the fatty acid composition and the total amount of derived fatty acid from each sample to be quantified. The identities of the peaks were verified by comparison with the retention times of standard fatty acid methyl esters.

To determine the antioxidant enzymes in the spermatozoa the cells were diluted by 0.01 M phosphate buffer, freeze-thawed twice and sonicated at 4°C for 3 min. After centrifugation the resultant supernatant was used for the assay.

GSH-Px activity was measured using a commercial kit-system (Randox, Crumlin, UK) by a coupled reaction with excess glutathione reductase and monitoring the NADPH oxidation at 340 nm. Total activity was determined using cumene hydroperoxide as a substrate and the activity of Se-dependent GSH-Px was measured with $H_2O_2$ as substrate [38].

Total SOD activity was determined using a commercial kit (Randox, Crumlin, UK), employing xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. A unit is defined as the quantity of SOD required to produce 50% inhibition of this reaction. To determine Mn-SOD the measurement was performed in the presence of 5 mM sodium cyanide [31].

The measurement of total antioxidant activity of seminal plasma was based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2[1]-azinobis (3-ethylbenzothiazoline 6-sulfonate) using Trolox as a standard and expressing results as $\mu$mol Trolox equivalents [37]. Total protein in the seminal plasma was determined by Bio-Rad Protein Assay.

Results are presented as mean $\pm$ S.E. of measurements on samples from seven to ten replicate determinations. Statistical analysis was performed using single factor ANOVA. Differences between means were evaluated at 5% level of significance.

Table 1
Fatty acid composition of the diets

| Fatty acid | Chicken | Turkey | Guinea fowl | Duck | Goose |
|---|---|---|---|---|---|
| 16:0 | 11.54 | 14.84 | 18.58 | 8.13 | 14.4 |
| 18:0 | 1.75 | 1.49 | 9.51 | 2.08 | 3.14 |
| 18:1n–9 | 31.13 | 31.84 | 31.73 | 45.3 | 19.90 |
| 18:1n–7 | 1.81 | 1.66 | ND | 2.40 | 1.18 |
| 18:2n–6 | 46.14 | 43.55 | 33.21 | 33.69 | 52.07 |
| 18:3n–3 | 4.43 | 3.44 | 1.68 | 5.82 | 4.39 |

Figures are percentage (w/w) of total fatty acids. Only fatty acids representing more than 0.5% are presented. ND, not detected.

## 3. Results

Fatty acid compositions of the diets are depicted in Table 1. Two major fatty acids 18:1n–9 and 18:2n–6 represent 65% (guinea fowl) and up to 79% (duck) of total dietary fatty acids. In general, the diets were very similar in containing low levels of linolenic (18:3n–3) acid, the precursor for n–3 PUFAs. The dietary level of $\alpha$-linolenic acid (18:3n–3) was lowest for the guinea fowl (1.68%) and highest for the duck (5.82%). The goose diet was characterised by comparatively low levels of 18:1n–9 and high concentrations of 18:2n–6.

The fatty acid composition of avian spermatozoa (Table 2) shows significant differences between the species studied. The main fatty acids of chicken spermatozoa were 22:4n–6, 18:0, 18:1n–9 and 20–4n–6; the PUFAs and saturated fatty acids represented 52.13 and 30.19% of total chicken sperm fatty acids, respectively. The main feature of turkey spermatozoa was their comparatively high levels of n–9 fatty acids (20:1n–9, 20:3n–9, 22:1n–9 and 20:3n–9) and relatively low concentrations of 22:4n–6. The levels of PUFAs in turkey spermatozoa were similar to those of goose spermatozoa and were lower than for the other species (46.62%). However, the turkey spermatozoa contained high levels of mono-unsaturated fatty acids (MUFAs) (21.28%). Compared to chicken spermatozoa, guinea fowl spermatozoa contained higher levels of 16:0 and 20:4n–6 and lower levels of 20:1n–9, 20:3n–6 and 22:4n–6, although the overall proportion of PUFAs and MUFAs (49.76 and 12.23%, respectively for guinea fowl) were similar in both species. Goose spermatozoa were characterised by the highest levels of 18:1n–9 and 18:2n–6 (13.66 and 5.68%, respectively) and their total content of PUFAs was lower compared to chickens although their MUFAs were of a similar level. The main characteristics of duck semen were relatively high levels of 22:6n–3 and comparatively high concentrations of 20:4n–6 and 16:0, but low levels of 18:0, 18:1n–9, and moderate concentrations of 22:4n–6. Duck spermatozoa had the highest (54.74%) levels of PUFAs and lowest concentration of MUFAs (9.20%).

Superoxide dismutase activity was detected in spermatozoa and seminal plasma from all avian species investigated here (Tables 3 and 4). The highest activity was recorded in goose and the least activity in chicken spermatozoa. More than 50% of the SOD activity in the chicken, turkey, guinea fowl and duck was KCN resistant: thus Mn-SOD is the main form of the enzyme in those species. On the other hand, in goose, Cu,Zn-SOD activity was higher than Mn-SOD.

In avian semen both forms of GSH-Px were found (Tables 4 and 5). Total GSH-Px activity in goose spermatozoa was the highest (178.7 $U/10^9$ cells); in guinea fowl it was more than 15 times lower ($11.83/10^9$ cells). In the species studied here, Se-GSH-Px represented from 77.7 (chicken) to 87.4% (guinea fowl) of total activity (Table 4). In general the total GSH-Px activity of water fowl spermatozoa was significantly higher compared to other species studied.

In seminal plasma, KCN inhibited 100% of SOD activity, signifying the presence of only Cu,Zn-SOD. In the seminal plasma, the highest SOD activity was found in turkey and guinea fowl (Table 4). On the other hand, in water fowl, SOD activity was low compared to other species studied. Seminal plasma SOD activities, expressed per mg protein, were ranked as guinea fowl > chicken > goose > duck > turkey.

Of seminal plasma GSH-Px activity, Se-GSH-Px represented 80% in chicken, 61% in turkey, 81% in duck and 82% in goose (Table 4). On the other hand, in guinea fowl seminal plasma, non-Se-GSH-Px was the main form of the enzyme, representing 62% of the activity. In general, water fowl seminal plasma GSH-Px activity was significantly higher than those of other species studied. Se-independent GSH-Px activity was the highest in turkey and guinea fowl being 2.4 times higher compared to chicken and almost five times higher compared to water fowl seminal plasma. The total specific activity of the GSH-Px expressed per mg of protein in seminal plasma was the highest in guinea fowl (79.16 mU $mg^{-1}$ protein) and the lowest in turkey (18.99 mU $mg^{-1}$ protein).

The total antioxidant potential of the seminal plasma of the avian species is shown in Table 6. Turkey seminal plasma was characterised by the highest antioxidant activity being more than 20 times higher than that of chicken. Duck and goose had similar seminal plasma

Table 2
Fatty acid composition of avian spermatozoa

| Fatty acid | Chicken | Turkey | Guinea fowl | Duck | Goose |
|---|---|---|---|---|---|
| 14:0 | <0.5 | <0.5 | 0.6 ± 0.1 | 1.1 ± 0.3 | 0.9 ± 0.2 |
| 14:1 | <0.5 | <0.5 | <0.5 | 0.8 ± 0.1 | <0.5 |
| 16:0 | 9.5 ± 0.6$^{a}$ | 12.4 ± 0.9$^{b}$ | 15.4 ± 0.8$^{b}$ | 21.5 ± 0.7$^{c}$ | 17.0 ± 2.0$^{b}$ |
| 17:0 | <0.5 | <0.5 | <0.5 | <0.5 | 0.6 ± 0.1 |
| 17:1 | <0.5 | <0.5 | 0.9 ± 0.3 | <0.5 | 1.1 ± 0.2 |
| 18:0 | 19.1 ± 0.9$^{a}$ | 17.7 ± 0.6$^{ab}$ | 15.8 ± 0.5$^{b}$ | 11.5 ± 0.1$^{d}$ | 15.4 ± 0.4$^{bc}$ |
| 18:1n–9 | 11.4 ± 0.1$^{a}$ | 7.7 ± 0.4$^{b}$ | 9.1 ± 0.3$^{bc}$ | 5.9 ± 0.2$^{e}$ | 13.7 ± 0.9$^{ad}$ |
| 18:1n–7 | 2.1 ± 0.1 | 2.5 ± 0.1 | 2.1 ± 0.1 | 2.1 ± 0.1 | 2.1 ± 0.2 |
| 18:2n–6 | 2.6 ± 0.2$^{a}$ | 4.6 ± 0.5$^{b}$ | 3.8 ± 0.3$^{abc}$ | 3.8 ± 0.4$^{abc}$ | 5.7 ± 0.4$^{bd}$ |
| 20:0 | 1.3 ± 0.2 | 1.2 ± 0.1 | 3.8 ± 1.2 | 1.1 ± 0.5 | 1.7 ± 0.1 |
| 20:1n–9 | 3.6 ± 0.2$^{a}$ | 9.2 ± 0.5$^{b}$ | 1.2 ± 0.2$^{c}$ | 0.7 ± 0.1$^{c}$ | 1.1 ± 0.1 $^{c}$ |
| 20:2n–6 | 1.5 ± 0.2$^{a}$ | 4.0 ± 0.9$^{ab}$ | 3.3 ± 0.8$^{ab}$ | 1.6 ± 0.2$^{a}$ | 3.9 ± 0.2$^{bc}$ |
| 20:3n–6 | 1.6 ± 0.1 | 0.9 ± 0.1 | <0.5 | 1.0 ± 0.1 | 0.5 ± 0.1 |
| 20:3n–9 | <0.5 | 2.7 ± 0.1$^{a}$ | 1.0 ± 0.1$^{b}$ | <0.5 | 0.9 ± 0.4$^{b}$ |
| 20:4n–6 | 11.7 ± 0.1$^{a}$ | 9.4 ± 0.2$^{b}$ | 15.8 ± 0.4$^{c}$ | 18.9 ± 0.2$^{d}$ | 13.3 ± 0.9$^{ac}$ |
| 22:1n–9 | <0.5 | 1.2 ± 0.1 | 0.7 ± 0.1 | <0.5 | <0.5 |
| 22:3n–9 | 3.8 ± 0.3$^{a}$ | 9.2 ± 0.4$^{b}$ | 4.3 ± 0.2$^{a}$ | 0.6 ± 0.1$^{c}$ | 1.4 ± 0.3$^{c}$ |
| 22:4n–6 | 27.9 ± 1.4$^{a}$ | 12.6 ± 0.6$^{b}$ | 19.4 ± 0.9$^{c}$ | 19.9 ± 0.8$^{c}$ | 17.5 ± 1.0$^{cd}$ |
| 22:5n–3 | 0.5 ± 0.1 | 0.7 ± 0.2 | <0.5 | 0.6 ± 0.1 | <0.5 |
| 22:6n–3 | 2.1 ± 0.5$^{a}$ | 2.7 ± 0.9$^{a}$ | 1.7 ± 0.3$^{a}$ | 8.0 ± 0.3$^{b}$ | 2.8 ± 0.5$^{a}$ |

Figures are mean ± S.E.M.percentage of total fatty acids. Numbers with superscripts are significantly different with respect to row.

antioxidant potential and guinea fowl had half of this activity. Turkey seminal plasma had the highest protein level and the guinea fowl seminal plasma had the lowest protein concentration.

## 4. Discussion

The fatty acid composition of chicken and turkey spermatozoa was similar to that found in previous studies [10,22–24,36,39]. With data from other species, reported here for the first time, it is now clear that in spermatozoa from all five avian species studied, the most significant PUFA is 22:4n–6, rather than 22:6n–3 as found in mammalian spermatozoa [25].

As can be seen from Table 1 species-specific differences in the C18 n–3 precursor ($\alpha$-linolenic acid) levels in the commercial diets used were not dramatic, although they were comparatively higher in the diet of the duck. On the other hand, the level of C18 n–6 precursor (linoleic acid) was highest in the diet of the goose and lowest in that of the guinea fowl, but the proportions of n–6 fatty acids in the spermatozoa of these species were reversed. These data clearly showed that species-specific differences in fatty acid composition of the spermatozoa in this study were more likely to be due to the genetic differences in the metabolism of fatty acids rather then differences in the dietary content of fatty acids. It would, however, be expected that the proportion of fatty acids in spermatozoa from these species would depend, to some extent, on the dietary provision of fatty acids, as has been shown for chickens [23,24].

Table 3
Superoxide dismutase activity in avian spermatozoa

| Species | Mn-SOD | Cu,Zn-SOD | Total |
|---|---|---|---|
| Chicken | 12.66 ± 1.47 | 9.38 ± 0.83 | 22.04 |
| Turkey | 10.81 ± 1.29 | 6.16 ± 0.85 | 16.97 |
| Guinea fowl | 15.12 ± 1.40 | 6.79 ± 0.83 | 21.91 |
| Duck | 35.10 ± 1.94** | 24.81 ± 2.25** | 59.91 |
| Goose | 22.40 ± 1.63* | 55.34 ± 4.23** | 77.74 |

Figures are mean ± S.E.M. activity as units per $10^9$ spermatozoa ($n = 7$). Asterisks represent significance of difference from chicken sperm activity: *$P < 0.05$; **$P < 0.01$.

Compared to other avian tissues [34], the relatively high levels of unsaturated fatty acids in spermatozoa, which varied from 46% in turkey up to 55% in drake, are likely to promote a susceptibility to lipid peroxidation. In general, duck spermatozoa had the highest levels of unsaturated fatty acids, but the increased potential susceptibility to peroxidation was apparently compensated by the presence of high levels of protective SOD and GSH-Px activities. Conversely, turkey spermatozoa had low levels of unsaturated fatty acids and low levels of SOD and GSH-Px. However, within the avian species studied here, the evidence for a relationship between the degree of unsaturation of sperm fatty acids and their activities of SOD and GSH-Px was not conclusive, since spermatozoa of the goose were ranked highest for antioxidant enzyme activities, but moderate for PUFA levels.

Table 4
Glutathione peroxidase and superoxide dismutase activity in avian seminal plasma

| Species | Se-dependent-GSH-Px | Non-Se-dependent GSH-Px | SOD |
|---|---|---|---|
| Chicken | 214.97 ± 14.09 | 53.66 ± 5.23 | 46.20 ± 2.44 |
| Turkey | 227.05 ± 11.07 | 145.15 ± 15.31xx | 77.71 ± 2.07* |
| Guinea fowl | 91.75 ± 7.34** | 148.89 ± 10.74** | 65.98 ± 1.54* |
| Duck | 110.84 ± 7.47** | 25.59 ± 2.82* | 32.27 ± 3.11* |
| Goose | 131.29 ± 10.13* | 27.88 ± 3.10* | 42.05 ± 3.06 |

All figures are mean ± S.E.M. activity ($n = 7$). GSH activities are U $l^{-1}$; SOD activities are as U $ml^{-1}$. Asterisks represent significance of difference from chicken sperm activity: *$P < 0.05$; **$P < 0.01$.

The distribution of SOD activity between Mn- and Cu,Zn-SOD in chicken and turkey spermatozoa was similar to that found in earlier reports [14] and of the same order as that found here for guinea fowl and duck. Goose spermatozoa, however, had particularly high levels of Cu,Zn-SOD. Total SOD activity in the different species was ranked goose > duck > chicken > guinea fowl > turkey. Chicken sperm SOD activity was similar [30], or, as with turkey levels, higher than that found in earlier reports [14]. These differences may be explained at least in part by different techniques used for preparation for analyses as well as different strains and age of poultry used. Se-GSH-Px was found to be the main form of the enzyme in spermatozoa from each species and the ranking of total sperm GSH activity was very similar to that of SOD, at goose > duck > chicken > turkey > guinea fowl.

The ranking of seminal plasma GSH and SOD activities were almost the reverse of their ranking in spermatozoa, although dissimilar to the total antioxidant activity of the seminal plasma. This may reflect the presence of other natural antioxidants in seminal plasma, like ascorbate, urate and albumin and to some extent glutathione and taurine [17] or other high and low molecular weight factors [26]. For example, the high protein concentration (Table 6) found in turkey seminal plasma may be related to its high antioxidant activity.

Superoxide radical formation is usually the result of electron leakage from uncoupled mitochondrial oxidative phosphorylation. Thus, a relationship between species differences in susceptibility to peroxidation and their rate of oxidative metabolism seems plausible. In chicken and turkey spermatozoa, the rate of oxidative metabolism is similar, although turkey spermatozoa have an absolute requirement for oxidative metabolism to maintain optimal ATP levels [41]. If this reflects greater demands on turkey sperm oxidative metabolism, then it may be significant that turkey spermatozoa have a lesser degree of fatty acid unsaturation compared to chicken spermatozoa, although they also have lower GSH and SOD activities. Unfortunately, no metabolic comparisons have been made between spermatozoa of the other avian species.

Whilst the importance of the proportion of PUFAs and susceptibility to peroxidation appear to be major factors in the survival of chicken and turkey spermatozoa during storage in vitro, they may also be factors in the survival of spermatozoa in vivo. One of the unique features of avian reproduction is the storage of spermatozoa within oviducal 'sperm storage tubules' [7], which enables the hen to produce fertile eggs during the 'fertile period' of 1–6 weeks, depending on the species [27]. Thus, avian spermatozoa might be expected to have systems which will maintain stability throughout this period. In this respect the levels of PUFAs in avian spermatozoa are less than those found in mammalian spermatozoa [25,32,35] and turkey spermatozoa have the least degree of fatty acid unsaturation and the longest fertile period. However, an antioxidant role of

Table 5
Glutathione peroxidase activity in avian spermatozoa

| Species | Se-dependent GSH-Px | Non-Se-dependent GSH-Px | Total |
|---|---|---|---|
| Chicken | 34.55 ± 2.77 | 9.91 ± 1.12 | 44.46 |
| Turkey | 25.46 ± 2.01 | 6.29 ± 0.72 | 31.75 |
| Guinea fowl | 10.34 ± 1.33** | 1.49 ± 0.26** | 11.83 |
| Duck | 58.71 ± 4.63* | 13.86 ± 2.11 | 72.57 |
| Goose | 151.16 ± 11.77** | 27.52 ± 3.36** | 178.68 |

Figures are mean ± S.E.M. activity as units per $10^9$ spermatozoa ($n = 7$). Asterisks represent significant difference from chicken sperm activity: *$P < 0.05$; **$P < 0.01$.

Table 6
Total antioxidant activity and protein levels in avian seminal plasma

| Species | AOA activity | Protein level |
|---|---|---|
| Chicken | 0.62 ± 0.02 | 4.69 ± 0.36 |
| Turkey | 13.15 ± 1.11** | 19.60 ± 1.12** |
| Guinea fowl | 0.97 ± 0.04* | 3.04 ± 0.106 |
| Duck | 2.10 ± 0.14** | 5.72 ± 0.48 |
| Goose | 2.13 ± 0.11** | 6.31 ± 0.53* |

All figures are mean ± S.E.M. activity ($n = 7$). Antioxidant activity is expressed as $\mu$mol Trolox equivalents per 1 ml; protein as mg $ml^{-1}$. Significance of difference from chicken: *$P < 0.05$; **$P < 0.01$; AOA, antioxidant activity.

the sperm storage tubules would have to be proposed, since enzymes such as SOD and GSH-Px are lower in turkey spermatozoa and, in general, lower in avian spermatozoa compared to mammalian spermatozoa [30].

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